AD_____

Award Number: DAMD17-02-1-0212

TITLE: Neurotoxicity From Chronic Exposure to Depleted Uranium

PRINCIPAL INVESTIGATOR: Stephen M. Lasley, Ph.D.

CONTRACTING ORGANIZATION: University of Illinois at Chicago
Chicago, IL 60612-7227

REPORT DATE: April 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	Apr 2003	Annual (1 Apr 2002-31 Mar 2003)

4. TITLE AND SUBTITLE
Neurotoxicity From Chronic Exposure to Depleted Uranium

5. FUNDING NUMBERS
DAMD17-02-1-0212

6. AUTHOR(S)
Stephen M. Lasley, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Illinois at Chicago
Chicago, IL 60612-7227

E-Mail: sml@uic.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

This project is designed to test the hypothesis that chronic exposure to depleted uranium (DU) impairs neuronal processes underlying cognitive function via alterations induced at hippocampal glutamatergic synapses. As prescribed by the Statement of Work, efforts were initiated in year 1 on Technical Objectives 1 (establishment of chronic exposure protocol) and 2 (defining integrity of hippocampal glutamate release) and are continuing into year 2. Blood and brain tissue are being collected in three exposure groups (0, 300, and 600 mg implanted DU pellets) across periods of 1-12 months in duration, and will be analyzed in two sample cohorts in year 2. A superfusion procedure has been successfully merged with liquid chromatography to measure stimulated hippocampal synaptosomal glutamate and GABA release in the presence and absence of Ca^{+2} (see Figures 1-2), and these experiments have begun utilizing acute exposure to uranyl ion. In addition, the component of Technical Objective 2 involving chronic exposure studies has been initiated by implantation of DU pellets in the majority of animals to be tested by intracerebral microdialysis. Thus, despite significant obstacles to progress the project is proceeding according to the Statement of Work schedule.

14. SUBJECT TERMS
Depleted uranium, glutamate release, military disease, NMDA receptor, AMPA receptor, hippocampus, microdialysis

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

The chemical properties of depleted uranium (DU) render the metal well suited for military purposes. The U. S. Army utilizes DU for tank armor and in munitions, and has deployed such weapons in the Gulf War, in Kosovo, and in Iraq. Use of the metal in future military arenas is a virtual certainty, but knowledge of its toxicity is lacking. Gulf War veterans who retained fragments of DU shrapnel over several years have exhibited lowered performance on neurocognitive tests (1). Moreover, research in chronically exposed rats has indicated alterations in hippocampal synaptic transmission, suggesting DU-induced decreases in neuronal excitability (2). *This research proposal will therefore test the overall hypothesis that chronic exposure to DU impairs neuronal processes underlying cognitive function via alterations induced at hippocampal glutamatergic synapses that directly modulate Ca^{+2}-mediated cellular processes.* Glutamatergic function will be assessed in rats exposed up to 12 months via intramuscular implants of varying amounts of DU pellets in order to identify the bases for the impaired cognition and diminished neuronal excitability. Components of depolarization-evoked glutamate release will be measured in the presence of acute *in vitro* or after extended *in vivo* exposure to the metal (Technical Objective 2). Determination of the actions of uranium on NMDA and AMPA receptors will be performed via approaches employing analogous *in vitro* and *in vivo* exposures (Technical Objective 3). Other studies will determine the concentrations of DU produced in blood and brain tissue as a result of exposure (Technical Objective 1). These results will be of critical importance to U. S. armed forces in defining risk and establishing treatment modalities for DU exposures sustained in recent conflicts and in future battlefield situations.

Body

As prescribed in the approved Statement of Work, project activities in year 1 addressed Technical Objectives 1 and 2. A description of these efforts and the resulting progress toward each Objective is provided below.

Technical Objective 1 concerned establishment of the chronic DU exposure protocol. Rats of approximately 60 days of age were exposed to 0 (controls), 300, or 600 mg DU by implantation of 30-mg pellets in the gastrocnemius muscles of their hindlimbs. Animals were anesthetized after exposure periods of 1, 3, 6, or 12 months (N = 6/group/time period), and 1 ml of blood collected by cardiac puncture and placed into heparinized tubes. The rats were then sacrificed and hippocampal brain tissue (~125 mg) dissected and weighed. At this point in time 85% of the study animals have been implanted with DU pellets, and blood and tissue samples have been collected from half (N = 3 of the 6 total) of the rats at the 1, 3, and 6 month durations. In year 2 samples will be submitted for neutron activation analysis performed by Element Analysis Corp. (Lexington, KY) in two cohorts equally representing all exposure groups and durations. This manner of sample submission was determined to be economical and statistically reliable. Attainment of Technical Objective 1 will be completed in year 2 of the project.

Technical Objective 2 concerned definition of the integrity of hippocampal glutamate release as a result of acute or chronic uranium exposure. In order to conduct the acute exposure studies a superfusion procedure was established that permitted measurement of endogenous glutamate and GABA release from hippocampal synaptosomes. A 10 mM HEPES buffer (containing in mM: Na^+ 133.2, K^+ 1, Mg^{+2} 1, PO_4^{-3} 1.2, Ca^{+2} 1.3, sucrose 10) saturated with O_2/CO_2 (95/5) and maintained at pH 7.4 was perfused at a rate of 0.6 ml/min through a 200 µl superfusion chamber containing 1 mg synaptosomal protein resting on a glass fiber filter. Release was stimulated with a 2-min perfusion of 31 mM K^+ (replacing Na^+ to maintain isotonicity) containing the glutamate reuptake blocker, 3-*threo*-hydroxyaspartate. Alternatively, Ca^{+2}-independent release is evoked by perfusing with high K^+ in Ca^{+2}-free buffer containing a voltage-sensitive Ca^{+2} channel blocker methoxyverapamil. An aliquot of each 2-min fraction of superfusate is derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and quantified by binary gradient liquid chromatography using a Waters' Breeze chromatography system (Waters Corp., Milford, MA) and fluorescence detection (excitation 250 nm, emission 395 nm). While it is more common to measure synaptosomal glutamate/GABA release employing ^3H-amino acids that are loaded into synaptosomes and then released by high K^+ stimulation, it is generally accepted that this form of release may not be drawn from the same intracellular pools as release of the endogenous transmitter. However, endogenous release is more analytically difficult to detect because of the small concentrations involved. The superfusion-liquid chromatography system described above possesses sufficient sensitivity to measure these latter concentrations.

Figure 1 displays a typical K^+-stimulated glutamate release from hippocampal synaptosomes using the system described above, exhibiting a ~15-fold increase in total (Ca^{+2}-dependent + Ca^{+2}-independent) release over baseline concentrations (each point is mean $\pm$ SEM of 4 replications). Also shown is the response to high K^+ occurring in the absence of Ca^{+2} in the perfusion medium (2 replications). It can be seen that this latter response constitutes 25-30% of the increase observed in the presence of Ca^{+2}. These distinctions in components of release have been fruitful in distinguishing the actions of exposure to other metals such as lead (3-4). Figure 2 displays endogenous GABA release in response to high K^+ stimulation, exhibiting ~7-fold increase in total release over baseline concentrations (6 replications). Experiments utilizing acute exposure to varying concentrations of uranyl ion in the perfusion medium are underway at this time. Analogous studies will be performed utilizing Pb^{+2} to serve as a positive control and to validate the sensitivity of the superfusion-chromatography system. These experiments will be completed in year 2 of the project.

Analogous experiments will be performed *in vivo* employing intracerebral microdialysis to quantify the changes in hippocampal glutamate/GABA release occurring after a chronic exposure period of 12 months. These experiments have been initiated with implantation of DU pellets in 65% of the study animals. These experiments will continue during year 2 of the project.

Despite the above project accomplishments, problems have been encountered that have significantly hindered further progress. Completion of the recruitment of a Chinese

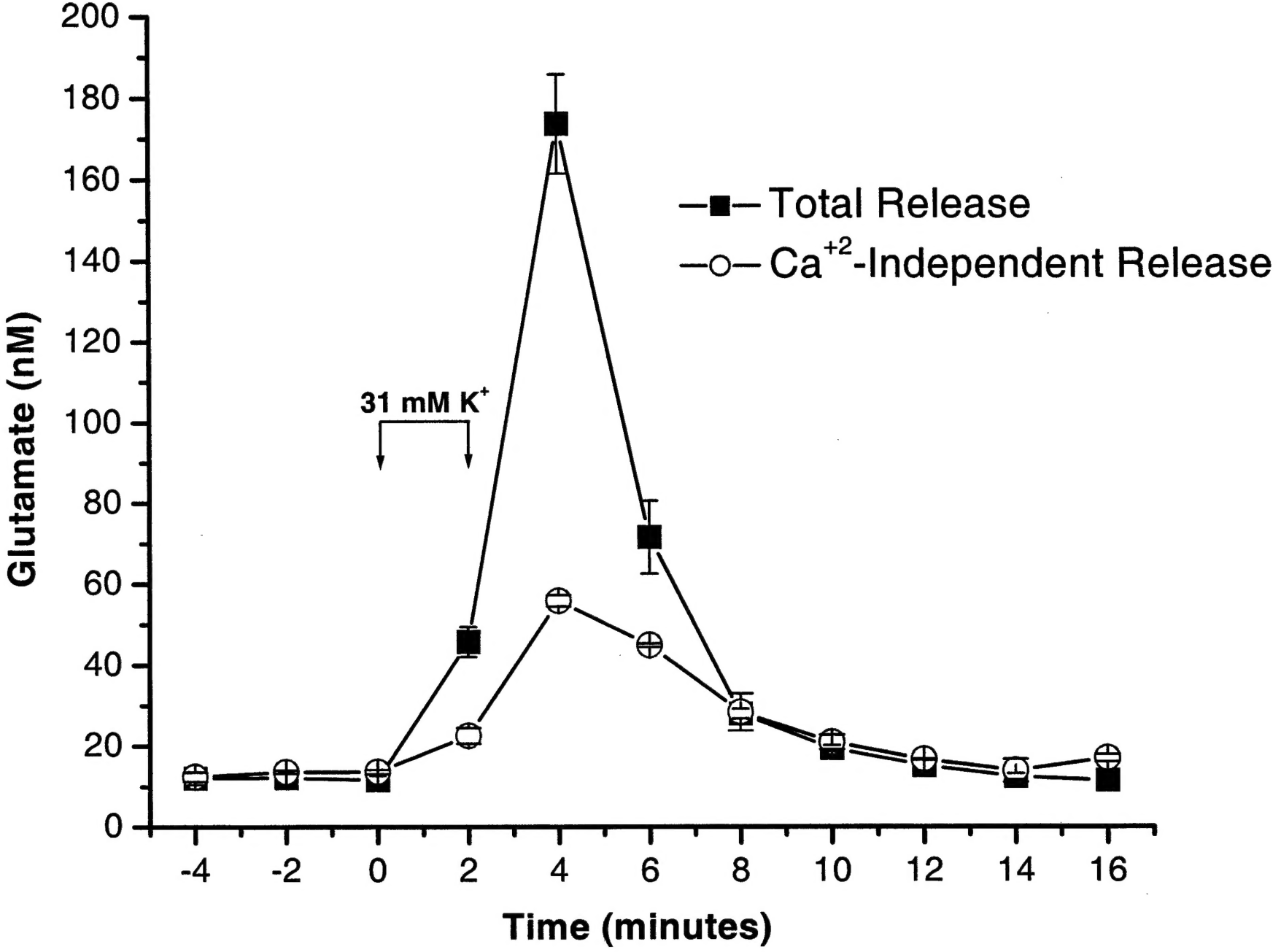
Glutamate (nM)
200
180
160
140
120
100
80
60
40
20
0
-4
-2
0
2
4
6
8
10
12
14
16
Time (minutes)
31 mM K+
Total Release
Ca+2-Independent Release

FIGURE 1

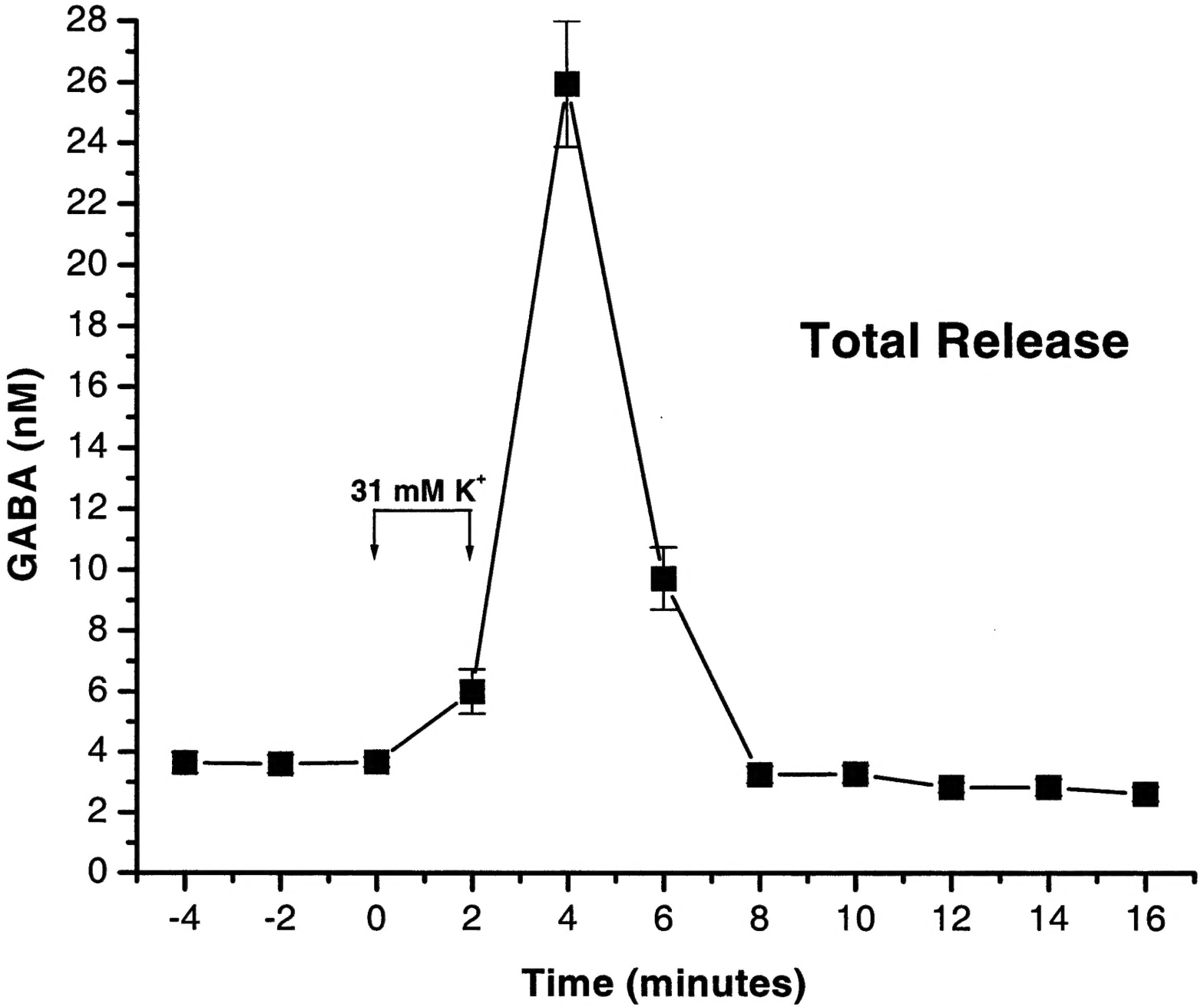
Total Release
GABA (nM)
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
31 mM K+
-4
-2
0
2
4
6
8
10
12
14
16
Time (minutes)

FIGURE 2

postdoctoral research associate has been delayed indefinitely by visa approval procedures. Currently, the candidate's H-1B application is awaiting clearance by the State Department in Washington, D.C. with no indication when approval will be forthcoming. Additional fees were paid to expedite the visa review procedures, but the process has been ongoing since the fall of 2002 with the candidate's application under State Department review since late February. A Bachelor's degree laboratory technician has been hired to temporarily replace the postdoctoral person, but this cannot compensate for the loss of professional skills and expertise.

Other problems have been directly associated with the acquisition and use of uranium. DU pellets are produced by vendors on a custom order basis and have required 3-5 months to be filled. The initial supply of pellets was ordered 6 weeks before the project start date of April 1, 2002, but was not delivered until July, thus causing an initial project delay. Also, to this point the DU product has varied greatly in price and finished quality. More recently, determination of acceptable disposal procedures for dilute uranium salt-containing solutions – such as are used in the acute exposure experiments – has posed significant problems. At this point in time it has not been determined whether these solutions can be flushed into the standard sewer system with appropriate monitoring, or whether they will have be shipped to a landfill as hazardous waste at considerable expense. The presence of acetonitrile in the chromatography system effluent further complicates the problem.

Key Research Accomplishments

Considerable effort has been invested to optimize the use of the superfusion-chromatography system for determination of endogenous synaptosomal glutamate and GABA release. These procedures will permit the most precise and reliable determination of the effects of acute exposure to uranyl ion on hippocampal transmitter release, experiments that are now underway.

Also, a surgical procedure for DU pellet implants has been developed that is efficient and reproducible, and has now been utilized in almost 100 rats. The efficiency of these procedures has resulted in recovery of >90% of the DU pellets after sacrifice of the animals, permitting disposal of the metal in a properly controlled fashion.

Reportable Outcomes

None at this time

Conclusions

Summaries of the results and statements of their importance as a scientific product are included in the preceding sections **Key Research Accomplishments** and also in **Body**. No conclusions on the effects of uranyl ion on neurotransmitter release can be stated at this time. The chronic DU exposure protocol will be established by analytical determinations to be conducted in the coming months.

References

1. McDiarmid, M.A., Keogh, J.P., Hooper, F.J., McPhaul, K., Squibb, K., Kane, R., DiPino, R., Kabat, M., Kaup, B., Anderson, L., Hoover, D., Brown, L., Hamilton, M., Jacobson-Kram, D., Burrows, B. and Walsh, M. Health effects of depleted uranium on exposed Gulf War veterans. *Environ. Res.* 82, 168-180 (2000).

2. Pellmar, T.C., Keyser, D.O., Emery, C. and Hogan, J.B. Electrophysiological changes in hippocampal slices isolated from rats embedded with depleted uranium fragments. *NeuroToxicology* 20, 785-792 (1999).

3. Lasley, S.M. and Gilbert, M.E. Rat hippocampal glutamate and GABA release exhibit biphasic effects as a function of chronic lead exposure level. *Toxicol. Sci.* 66, 139-147 (2002).

4. Lasley, S.M., Green, M.C. and Gilbert, M.E. Influence of exposure period on *in vivo* hippocampal glutamate and GABA release in rats chronically exposed to lead. *NeuroToxicology* 20, 619-630 (1999).

Appendices

None